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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Kasahara et al. Art Unit : 1633
Serial No.: 10/043,912 Examiner : David Guzo
Filed : January 11, 2002
Title : GENE DELIVERY SYSTEM AND METHODS OF USE

Commissioner for Patents
Washington, D.C. 20231

DECLARATION OF NORIYUKI KASAHARA
UNDER 37 C.F.R. §1.132

Dear Sir:

1. I, Noriyuki Kasahara, declare and say I am a resident of Los Angeles, California. My residence address is 8446 Kirkwood Drive, Los Angeles, California 90046.

2. I, Noriyuki Kasahara, hold a Bachelor degree in Medical Science that I received from Tokyo Medical and Dental University, in 1986. I further hold an M.D. degree that I received from Tokyo Medical and Dental University in 1993, and a Ph.D. degree that I received from the University of California, San Francisco (UCSF) in 1994. I received Board certification in the field of Clinical Pathology from the American Board of Pathology in 1996. I am currently an Associate Professor in the Department of Medicine at the University of California, Los Angeles (UCLA) David Geffen School of Medicine, 675 Charles E.

CERTIFICATE OF MAILING BY FIRST CLASS MAIL

I hereby certify under 37 CFR §1.8(a) that this correspondence is being deposited with the United States Postal Service as first class mail with sufficient postage on the date indicated below and is addressed to the Commissioner for Patents, Washington, D.C. 20231.

December 9, 2003
Date of Deposit

Teri Barnett
Signature

Teri Barnett
Typed or Printed Name of Person Signing Certificate

Young Drive South, MRL-1551, Los Angeles, CA. I am an expert in the fields of clinical pathology, molecular biology, virology, virus-based gene delivery vector technology development, and gene therapy.

3. I am an inventor of the claims of the above-identified patent application. I directed others and personally performed the research leading to the invention disclosed and claimed therein.

4. I have read the Office Action dated September 10, 2003, issued for the above-identified application. I understand that the Examiner has rejected the pending claims on the grounds that the specification allegedly fails to provide an enabling disclosure for methods of treating a mammal having a cell proliferative disorder with a replication competent retrovirus. The Examiner alleges that the art of gene therapy is unpredictable and poorly developed.

5. The specification of the above-referenced patent application describes replication-competent retrovirus (RCR) vectors. These vectors are unique, in part, because their design greatly enhances genetic and functional stability of the vector. All previously reported replication-competent vectors have contained transgene insertions within the 3' long terminal repeat (LTR) which were invariably deleted within 1 or 2 serial passages. To alleviate this problem, we inserted an internal ribosome entry site (IRES) sequence and multiple cloning site into a less sensitive position in the viral genome that linked transgene expression to viral coding sequences. The IRES-

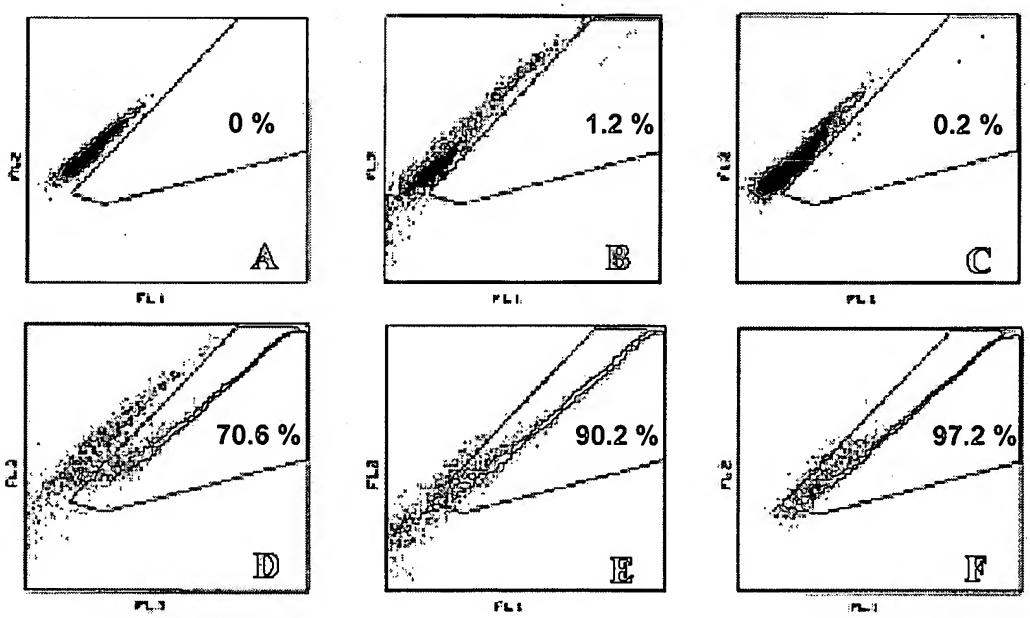
multiple cloning sequence is positioned between the env termination codon and a polypurine-rich tract within the 3' untranslated region (UTR) that binds reverse transcriptase. The cloning site is useful for insertion of transgene coding sequences, including marker genes such as green fluorescent protein (GFP), suicide genes such as yeast cytosine deaminase (CD) or purine nucleoside phosphorylase (PNP), and expression of each of these transgenes is linked to viral gene expression through the IRES sequence. There have been no previous reports regarding use of an IRES sequence to link transgene expression with viral gene expression in MLV-derived replication-competent retrovirus vectors; hence this represents a novel design strategy. The ACE-GFP RCR vector described in parts 6 and 7 of the present declaration is the same as the vector described in Example 15 of the pending application. The ACE-CD RCR vector described in part 8 of the present declaration is similar to the ACE-PNP vector described in Example 19 of the pending application, except that cytosine deaminase (CD) has been substituted for purine nucleoside phosphorylase (PNP).

6. The previously described RCR vectors achieve efficient transgene delivery to solid tumors in vivo. U-87 human glioma cells (5×10^5 cells) were first implanted subcutaneously into athymic nu/nu mice. Tumors were allowed to grow up to 0.5 cm in diameter (at which point there are 10^{6-7} total glioma cells in the tumor mass), then PBS vehicle control, the amphotropic RCR vector ACE-GFP (1.2×10^5 TU/100 µl), or a conventional replication-defective retrovirus vector expressing GFP (1.0×10^5 TU/100 µl) was injected into the tumor. After sacrifice at serial time intervals 2, 4, and 6 weeks after

vector inoculation, quantitation of GFP expression in the transduced tumors was performed by FACS analysis immediately after dissection and collagenase digestion to obtain a single-cell suspension of the tumor sample.

FACS analysis of replication-defective vs. RCR vector-transduced subcutaneous gliomas is provided in Figure 1. The percentage of GFP positive cells in the freshly dissected tumors that had been infected by the replication-defective vector was low, only 1.2% at 2 weeks, and 0.2% at 6 weeks post-vector injection (Figure 1, B-C), consistent with the results observed in clinical trials. In contrast, the percentage of GFP positive cells infected by the replication-competent retrovirus (ACE-GFP) was 70.6%, 90.2%, and 97.2% at 2, 4, and 6 weeks post-injection, respectively (Figure 1, D-F). These results demonstrate that, in contrast to conventional replication-defective vectors, the RCR vector was capable of essentially complete transduction of the entire U-87 tumor mass within 6 weeks.

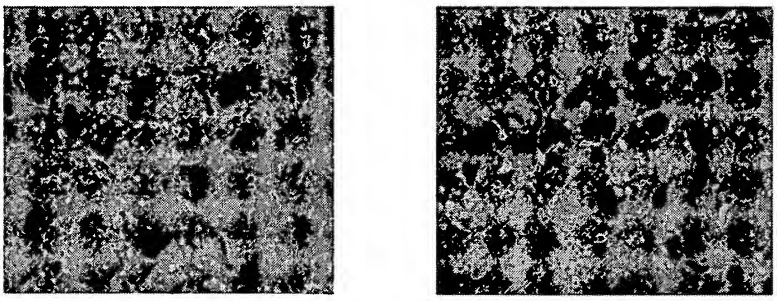
Figure 1



7. We have further tested the RCR vector for infectivity of non-target tissue. We have found that, even when directly injected into normal rat brain, the vector fails to transduce quiescent normal cells. The MLV-based RCR vector (ACE-GFP) (1.2×10^4 TU/10µl) was injected directly into the right frontal lobe of normal mice, and GFP expression assessed by immunohistochemistry. No GFP signal was detected in normal brain tissue injected with ACE-GFP (Figure 2) (A) Section of normal brain injected with PBS, or (B) ACE-GFP (1.2×10^4 TU) into the right frontal lobe of euthymic mice. Both sections were processed for GFP immunohistochemistry. These results demonstrate that the MLV-based RCR vector is completely incapable of infecting quiescent non-dividing normal brain tissue, but as shown above, the vector shows extremely efficient gene transfer to actively dividing tumor cells.

Figure 2

A **B**

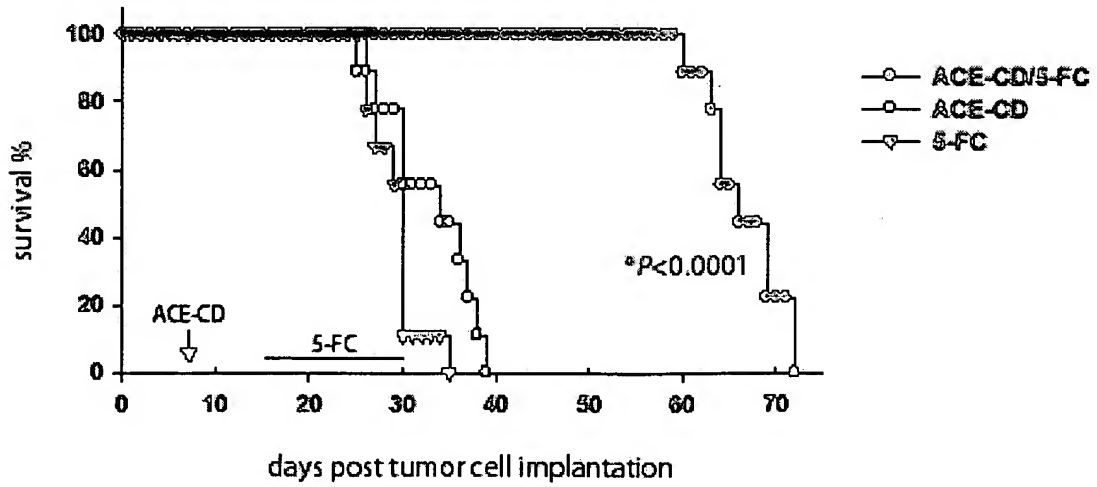


8. We have further found that methods employing the novel replication competent retrovirus significantly prolong survival of athymic mice implanted with U-87 intracerebral gliomas. An RCR vector expressing the yeast cytosine deaminase

suicide gene was tested in the intracranial U-87 glioma model. Yeast cytosine deaminase converts the non-toxic pro-drug 5-fluorocytosine (5-FC) to the toxic metabolite 5-fluorouracil (5-FU), which acts as an intracellular chemotherapeutic agent to kill transduced tumor cells.

One week after tumor implantation, approximately 1.0×10^4 TU in a total volume of 10 µl was stereotactically injected into intracranial U-87 tumors in 2 groups of mice (n = 9 each). An additional group (n = 9) received only PBS vehicle control. Eight days after vector transduction, the 5-FC prodrug, 500 mg/kg/day, was given for 15 consecutive days by daily intraperitoneal injection to one of the ACE-CD injected groups and to the PBS vehicle injected group. The remaining ACE-CD injected group received only daily intraperitoneal injections of PBS for 15 consecutive days. The mice treated with ACE-CD plus a single cycle of 5-FC prodrug showed a doubling of median survival over a follow-up period of more than 70 days (See Figure 3), compared to mice treated with either ACE-CD/PBS ($p<0.0001$) or PBS/5-FC prodrug ($p<0.0001$).

Figure 3



9. The specification of the above-referenced patent application provides ample guidance to the person of ordinary skill in the art to successfully practice the claimed invention. For example, one skilled in the art at the time the application was filed would know, given the information provided in the specification, that treatment of a subject with an RCR vector of the invention would likely result in a therapeutic effect.

10. All of the experiments described in the specification, in conjunction with above experiments which were performed according to methods and the examples described in the specification, demonstrate that the claimed invention is useful for efficiently transferring a therapeutic polypeptide to a large number of neoplastic cells in vivo. The experiments further indicate that, post-infection, the RCR vector produces therapeutic levels of a pro-drug in a subject. Using the methods and compositions described in the specification, one of ordinary skill in the art would have a reasonable expectation that the claimed invention would be applicable to providing a expression of a pro-drug in neoplastic cells for the purpose of treating a cell-proliferative disorder in a subject in need of such treatment.

11. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title XVIII of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 12/5/03

Noriyuki Kasahara, M.D., Ph.D.

Fish & Richardson P.C.
12390 El Camino Real
San Diego, CA 2130-2081
Telephone: (858) 678-5070
Facsimile: (858) 678-5099